

**DATA EVALUATION RECORD  
HEALTH MONITORING - HONEYBEES  
(NON-GUIDELINE STUDY)**

1. **CHEMICAL:** Clothianidin PC Code No.: 044309
2. **TEST MATERIAL:** Poncho (not further described) Purity: Not reported.

3. **CITATION**

Authors: Federal Department of Economic Affairs FDEA; Federal  
Office of Agriculture FOAG; Pesticide Department  
Switzerland

Title: Bee Health Monitoring in Switzerland (Translation)

Study Completion Date: September 10, 2009

Laboratory: Bayer CropScience  
2 T.W. Alexander Drive  
Research Triangle Park, North Carolina

Sponsor: The Swiss Agriculture Authorities  
Switzerland

Laboratory Report ID: G202097

MRID No.: 478817-01

DP Barcode: 374484

4. **REVIEWED BY:** Joan Gaidos, Senior Scientist, Cambridge Environmental, Inc.

**Signature:** 

**Date:** 7/15/11

**APPROVED BY:** John Marton, Staff Scientist, Cambridge Environmental, Inc.

**Signature:** 

**Date:** 8/1/11

5. **APPROVED BY:** {.....}, {Specialty}, OPP/EFED/ERB-{Section}

**Signature:**

**Date:**

## 6. STUDY PARAMETERS

**Test Species:** Honeybees (species and genus not specified). Healthy colonies were used, 20,000 and 12,000 bees per colony (species not reported) were set up on two sides of the corn fields for the first and second trial, respectively, (6 bee colonies per trial) and placed in fallow strips directly adjacent on two sides to seed-treated corn and flight entrances for colonies facing the corn field.

**Age of Test Organism at Test Initiation:** Not specified. Further details of the queen and the mix of sex and age were not reported.

**Test Duration:** Approximately 6 weeks

## 7. CONCLUSIONS:

In two separate field trials over approximately 6 weeks in Switzerland, 6 bee colonies (three per side) were placed next to a field sowed with clothianidin-treated corn seedlings dressed with the formulation Poncho at a rate of 25 g a.i./50,000 corn seeds. The study was designed as background information to determine if the defined dressing quality and the use of deflectors were sufficient to minimize the drift of toxic dust/abrasion particles so that bees and bee colonies are not indirectly harmed and to determine if guttation fluid represents a risk to the health of bee colonies.

In the first trial, the mean mortality rate ( $n = 4$ ) was 3-12 bees/day prior to sowing, 9-45 bees/day between days 1-3, and 17-21 bees/day between week 1 and week 6. The difference between colonies over time was 8 to 130%. In the second trial, the mean mortality rate ( $n = 4$ ) was 5-10 bees/day prior to sowing, 7-20 bees/day between days 1-3, and 10-20 bees/day between week 1 and week 6. The difference between the colonies over time was 40 to 120%.

Clothianidin residues were not detected in any of the bee or honey samples. In the pollen, clothianidin residues were below the limit of detection (0.004 mg/kg) with the exception of one pollen sample from day 1 following sowing in the first trial, which was 0.079 mg clothianidin/kg pollen. Concentrations of clothianidin in the guttation fluid varied from 25,000 to 37,000  $\mu\text{g/L}$ .

The study authors reported that increased bee deaths did not occur during the trial, though the mortality of one hive was slightly higher during at 1-3 days following sowing at  $45 \pm 23$  bees/day, compared to the period prior to sowing at  $12 \pm 8$  (statistical methods not described). For comparison, the mean death rate of a colony in the spring is about 1500 bees/day (reference not reported); however, the majority (not defined) die in the field and cannot be detected by the method of counting dead bees around the flight entrance (reference not reported). Regular observation (not defined) indicated that bee colonies developed normally during the trials; however, specific population measurements of test colonies were not determined. The single positive clothianidin residue value in the pollen (0.079 mg/kg) was determined to have no effect

on the development of the bee colonies (method of determination not described). According to the study authors, the finding of clothianidin in pollen in the first trial can be explained due to a corn variety demonstration trial occurring at the same time and an increase in flowering dandelions is conceivable due to the frequent change of corn seeds at the margins of the field.

The guttation fluid contained high concentrations of clothianidin (between 25,000 and 39,000 µg/L), that fell with increased growth of the corn plants (tabular data not reported). However, the risk to bees remained present for several weeks. If the concentrations in the guttation fluid are compared to the acute lethal doses of orally applied active substance (oral LD<sub>50</sub>: 3.94 and 3.79 ng clothianidin after 24 and 48 hours, respectively; Bayer Dossier, Weyman 1998), a concentration of 95 µg/L in guttation fluid corresponds to the acute oral LD<sub>50</sub>, assuming a water carrier can ingest about 40 µL. Up to about 40 days after sowing, the guttation fluid contained clothianidin concentrations in the critical range. The study author concluded that since the mortality rates differing from the reference rates did not occur during weeks 1 through 6, and no clothianidin residues were detected in the bee or honey, it was assumed that guttation fluid was not ingested under the conditions of the trial. Supporting tabular data, detailed analytical methods, statistical analysis performed, and other pertinent details were not reported.

The reviewer concludes that the data presented in this study are inadequate to accurately determine the effects of clothianidin-treated corn seeds on bees and bee colonies or address the aims of the study: to verify the current regulation that the use of deflectors is sufficient to minimize the drift of toxic dust/abrasion particles so that bees and bee colonies are not directly harmed; and to verify the risk to bees and bee colonies through guttation fluid.

The study was conducted for 6-weeks, seed treatment and test formulation details were not described, test organisms were not described, analytical methods were not reported, statistical analysis were not described, controls were not used, environmental conditions were not adequately described, the efficacy of the endpoints and the methods for determining the endpoints were not validated, criteria for determining effects were not detailed, and tabular data was not presented to allow independent analysis. Furthermore, the study authors confirmed that concentrations of clothianidin residues measured in the guttation fluid were within the critical range corresponding to the acute LD<sub>50</sub> for up to about 40 days. Also, in the first trial, corn treated with other test material (Mesurol, Cruiser or Gaucho) was evidently planted at the same time as the clothianidin-treated corn seed, confounding and invalidating the results of the first trial.

The study authors presented that the “majority” of bees die in the field, not at the flight entrance; however, the percentage represented by the “majority” is not defined, nor is a reference for this detail provided. Additionally, the study authors stated that the concentrations of clothianidin residues measured in the guttation fluid were within the critical range corresponding to the acute LD<sub>50</sub> (citation provided) for up to about 40 days. Considering there were other food sources

nearby (dandelion, apple trees, and clover), combined with the majority of bees likely dying in the field (not sampled or estimated), the low bee mortality rates determined and the absence of clothianidin residues in dead bees collected at the flight entrance is not an accurate indicator of bee mortality due to exposure to clothianidin because of these confounding factors. Finally, if bees died rapidly in the fields due to clothianidin exposure, they may not have returned to the hive to contaminate the honey, and therefore clothianidin would not be found in the honey. The speed of bee death following exposure to guttation fluid within the acute LD<sub>50</sub> range was not explored or reported. Although the study author states that colony development was not affected, no specific population measurements of the test colonies were performed and therefore, colony health could not be determined.

## 8. **ADEQUACY OF THE STUDY**

**A. Classification:** Core/Supplemental/Invalid

**B. Rationale:**

**C. Repairability:**

9. **GUIDELINE DEVIATIONS:** This is a non-guideline test.

10. **SUBMISSION PURPOSE:** This study was submitted to verify current regulation during corn sowing and the risk to bees and bee colonies through guttation fluid. The study was designed as background information to determine if the defined dressing quality and the use of deflectors were sufficient to minimize the drift of toxic dust/abrasion particles so that bees and bee colonies are not indirectly harmed and to determine if guttation fluid represents a risk to the health of bee colonies. The test material, clothianidin, was applied as a seed treatment at a rate of 25 g a.i./50,000 corn seeds as the formulation Poncho.

## 11. **MATERIALS AND METHODS**

### **Test Material**

The test material was Poncho for seed treatment and contained the active ingredient clothianidin at 25 g a.i./50,000 corn seeds; no further details were reported.

### **Test Organisms**

Three bee colonies with *ca.* 20,000 and 12,000 bees per colony (species not reported) were set up on two sides of the corn fields for the first and second trial, respectively, (6 bee colonies per trial) and placed in fallow strips directly adjacent on two sides to seed-treated corn and flight entrances for colonies facing the corn field.

### **Seed Treatment and Crop Maintenance**

Two fields of corn were planted with Poncho-treated seeds on April 23, 2009 and May 17, 2009 using a sowing machine fitted with a deflector. In the first trial, the plants flowering in neighboring strips were dandelions and apple trees. Weather conditions were good with no rain the day of sowing, the average wind speed was *ca.* 2.6 m/sec peaking at 10.2 m/sec. The average temperature was 11°C (5.5-17°C). Bee colonies 48, 84 and 88 were located downwind during sowing and colonies 11, 33 and 56 were upwind. In the second trial, the plants flowering in the marginal strips were dandelion and clover. On the day of sowing, it rained (19.9 mm), average wind speed was 1.6 m/sec peaking at 11.9 m/sec, the average temperature was 15°C (7.2-22°C). The two fields were separated by about 200-300 m from each other. No further details of crop maintenance, seed variety, etc, were provided.

### Test Design

The experiments were conducted under field conditions in the INFORAMA Rütli agricultural college in Zollikofen. Two fields of corn were planted using Poncho-treated seeds. In the first trial, a corn field of 2 ha was sown on April 23, 2009 with Poncho-treated seeds over an area equivalent to 36% of the field. The remaining area was sown with corn varieties dressed with Mesurol, Cruiser or Gaucho. In each case, three bee colonies of 20,000 bees per colony were set up 6 days prior to sowing in a fallow strip directly adjacent on two sides by the corn field in which eight rows of corn bordering the strips were sown. In the second trial, a corn field of 1 ha was sown on May 17, 2009 with Poncho-treated seeds (area not reported). Seeds were sown directly into a mowed meadow sprayed with herbicide (formula not reported) in rows tilled by the sowing machine. Three bee colonies were set up in a meadow immediately adjacent on two sides of the corn field 16 days prior to sowing. Hives were heavily populated with about 12,000 bees per colony and 4 honeycombs with broods.

For both trials, two of the three bee colonies per side of each corn field were used to determine bee mortality using Münster traps and clothianidin residues in dead bees (total of 8 bee colonies). Pollen was collected from a pollen trap at the flight entrance of the third bee colony and tested for clothianidin residues (total of 4 bee colonies). Honey samples were collected before and 3-5 weeks after sowing and examined for clothianidin residues. During the early growth phase of the corn, guttation fluid was collected from plants in early morning and analyzed for clothianidin residues.

The first trial was conducted for a total of 50 days from the time of sowing (April 17, 2009) to the end of sampling (June 8, 2009). Bee mortality was determined at -3, -2, -1, and 0 days prior to sowing, then daily for the first week, and 3 days in the week until trial end. Pollen was collected from 2 of the colonies on -3 days prior to sowing, immediately after sowing and thereafter, with residues analyzed on 0, 1, 2, 3, 5, 7, 9, and 11 days. Honey was collected from all colonies on day 0 prior to sowing and at 19 days. Guttation

fluid samples were collected at 15, 20, 25, 27, 29, 32, 34, 41, 43, 46, 47, 48, 49 and 50 days.

The second trial was conducted for a total of 38 days from the time of sowing (May 17, 2009) to the end of sampling (June 24, 2009). Bee mortality was determined almost daily from -16 days prior to sowing, then daily to day 9, and 3 days in a week until trial end. Pollen was collected from all colonies from 2 colonies on -7, -5, and -2 days prior to sowing, immediately after sowing and thereafter, with residues analyzed on 0, 1, 5, 7, and 9 days. Honey was collected from all colonies 12 days prior to sowing and 38 days after sowing. Guttation fluid was collected at 8, 9, 10, 17, 19, 22, 24, 26, 31, 33 and 38 days.

### **Residue analysis**

Clothianidin residues were analyzed in bees, pollen, honey and guttation fluid samples collected over approximately 8 weeks after sowing clothianidin-treated corn seeds. Analysis was performed using LC-MS/MS method with a detection limit of 0.25 ng/bee, 0.004 mg/kg for pollen, 0.010 mg/kg for honey, and 0.10 µg/L for water. Analysis was performed by Harian Laboratories, Itingen. No further details of analysis methods were provided.

## **12. REPORTED RESULTS**

Signed and dated No Data Confidentiality and GLP statements were provided; a Quality Assurance statement was not provided. This study was not conducted in compliance with the Principles of Good Laboratory Practice (GLP). The study was designed as background information to determine if the defined dressing quality and the use of deflectors sufficient to minimize the drift of toxic dust/abrasion particles so that bees and bee colonies are not indirectly harmed and to determine if guttation fluid represents a risk to the health of bee colonies.

The period before sowing was evaluated as the reference values, while the first three days after sowing included the potential for acute effects on the bees. The data from these two periods are intended to show whether the current regulations governing sowing dressed corn seeds ensure acceptable risk to bees. The period from week 1 through week 6 included the potential risk to bees due to guttation water from young corn plants.

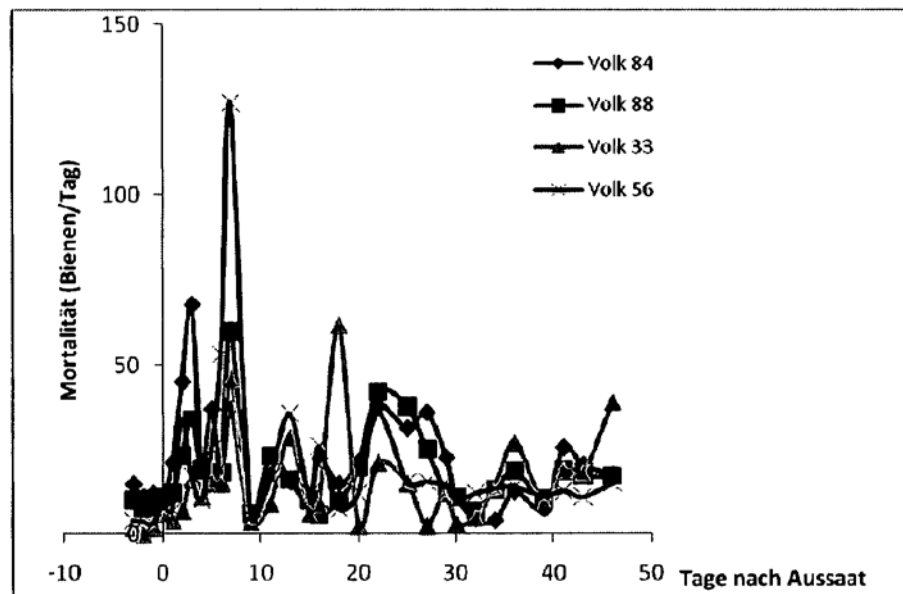
In the first trial, the mean mortality rate ( $n = 4$ ) was 3-12 bees/day prior to sowing, 9-45 bees/day between days 1-3, and 17-21 bees/day between week 1 and week 6. The difference between colonies over time was 8 to 130%. In the second trial, the mean mortality rate ( $n = 4$ ) was 5-10 bees/day prior to sowing, 7-20 bees/day between days 1-3, and 10-20 bees/day between week 1 and week 6. The difference between the colonies over time was 40 to 120%.

Mortality rates in the first trial (means  $\pm$  standard deviations)

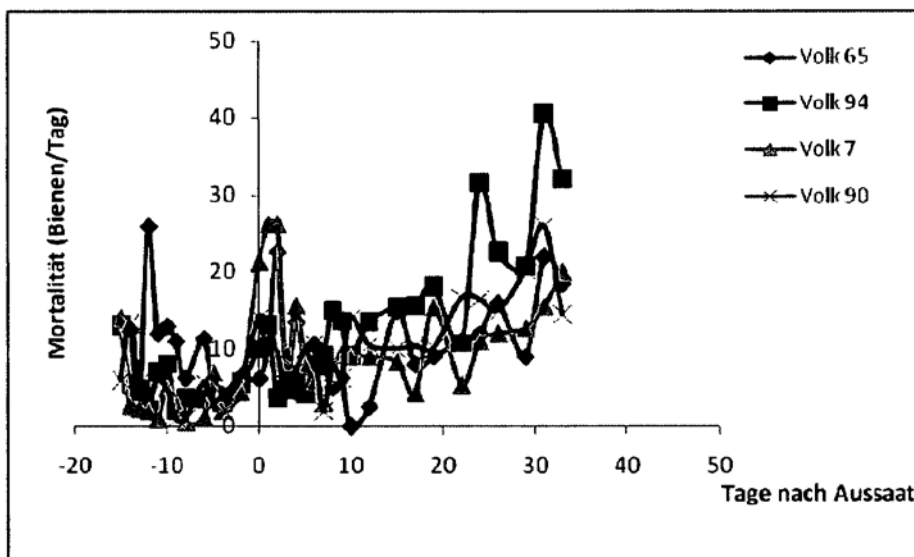
	Mortality (bees/day): mean $\pm$ SD			
Time	Colony 84	Colony 88	Colony 33	Colony 56
Before sowing	12 $\pm$ 8	9 $\pm$ 1	3 $\pm$ 1	5 $\pm$ 1
After sowing (1-3 days)	45 $\pm$ 23	23 $\pm$ 11	9 $\pm$ 6	19 $\pm$ 14
After sowing (1-6 weeks)	17 $\pm$ 14	19 $\pm$ 14	18 $\pm$ 22	21 $\pm$ 23

Mortality rates in the second trial (means  $\pm$  standard deviations)

	Mortality (bees/day): mean $\pm$ SD			
Time	Colony 65	Colony 94	Colony 7	Colony 90
Before sowing	10 $\pm$ 6	5 $\pm$ 3	5 $\pm$ 6	6 $\pm$ 4
After sowing (1-3 days)	13 $\pm$ 8	7 $\pm$ 5	20 $\pm$ 10	9 $\pm$ 4
After sowing (1-5 weeks)	10 $\pm$ 6	20 $\pm$ 9	11 $\pm$ 4	14 $\pm$ 5

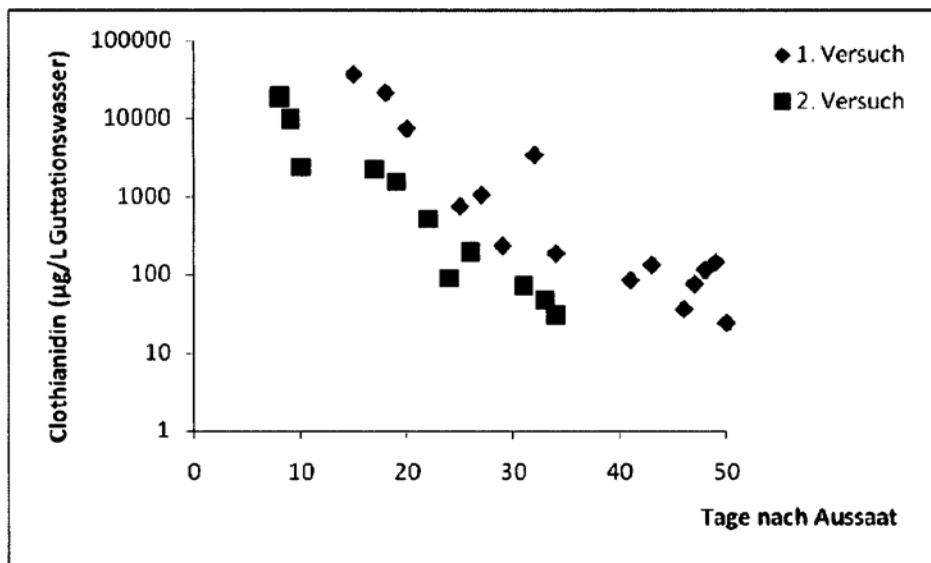
Bee mortality in the first trial: y-axis = mortality (bees/day) and x-axis days after sowing

Bee mortality in the second trial: y-axis = mortality (bees/day) and x-axis = days after sowing (misabeled in the MRID)



Clothianidin residues were not detected in any of the bee or honey samples. In the pollen, clothianidin residues were below the limit of detection (0.004 mg/kg) with the exception of one pollen sample from day 1 following sowing in the first trial, which was 0.079 mg clothianidin/kg pollen. Concentrations of clothianidin in the guttation fluid varied from 25,000 to 37,000 µg/L.

Clothianidin concentration in guttation fluid in the first and second trials: y-axis = clothianidin (µg/L guttationswasser) and x-axis = days after sowing



The study authors reported that increased bee deaths did not occur during the trial, though the mortality of one hive was slightly higher during at 1-3 days following sowing at  $45 \pm 23$  bees/day, compared to the period prior to sowing at  $12 \pm 8$  (statistical methods not described). For comparison, the mean death rate of a colony in the spring is about 1500 bees/day (reference not reported); however the “majority” (not defined) die in the field and cannot be detected by the method of counting dead bees around the flight entrance (reference not reported). Regular observation (not defined) indicated that bee colonies developed normally during the trials; however, specific population measurements of test colonies were not determined. The single positive clothianidin residue value in the pollen (0.079 mg/kg) was determined to have no effect on the development of the bee colonies (method of determination not described). According to the study authors, the finding of clothianidin in pollen in the first trial can be explained due to a corn variety demonstration trial occurring at the same time and an increase in flowering dandelions is conceivable due to the frequent change of corn seeds at the margins of the field. Therefore, since this is not normal practice, it can be assumed there is no risk to bee under normal circumstances, but does show that clothianidin-treated corn seeds must be used carefully in areas of flowering honey plants.

The guttation fluid contained high concentrations of clothianidin (between 25,000 and 39,000 µg/L), that fell with increased growth of the corn plants (tabular data not reported). However, the risk to bees remained present for several weeks. If the concentrations in the guttation fluid are compared to the acute lethal doses of orally applied active substance (oral LD<sub>50</sub>: 3.94 and 3.79 ng clothianidin after 24 and 48 hours, respectively; Bayer Dossier, Weyman 1998), a concentration of 95 µg/L in guttation fluid corresponds to the acute oral LD<sub>50</sub>, assuming a water carrier can ingest about 40 µL. Up to about 40 days after sowing, the guttation fluid contained clothianidin concentrations in the critical range. The study author concluded that since the mortality rates differing from the reference rates did not occur during weeks 1 through 6, and no clothianidin residues were detected in the bee or honey, it was assumed that guttation fluid was not ingested under the conditions of the trial.

Supporting tabular data, detailed analytical methods, statistical analysis performed, and other pertinent details were not reported.

The study author noted that the results suggest that there would be an increased risk to the bees if water sources are limited in the immediate vicinity. Therefore, good apiary practice of making a source of water available is to be taken seriously.

The study author concluded:

- In both trials unnatural bee mortalities did not arise immediately after sowing and no clothianidin residues were detected in the bees, confirming that under practical conditions with moderately flowering plants in the neighbourhood of the sowing operation. Therefore, the current regulations on the application of clothianidin-treated corn seeds are adequate given adherence to regulations are strictly monitored.
- Increased bee mortality did not occur in either trial during the guttation period and clothianidin residues were not detected in the bees or honey. Harm to the health of the bee colonies are excluded from the conditions chosen for the trials.

### **13. REVIEWER'S COMMENTS**

The reviewer concludes that the data presented in this study are inadequate to accurately determine the effects of clothianidin-treated corn seeds on bees and bee colonies or address the aims of the study: to verify the current regulation that the use of deflectors is sufficient to minimize the drift of toxic dust/abrasion particles so that bees and bee colonies are not directly harmed; and to verify the risk to bees and bee colonies through guttation fluid.

The study was conducted for 6-weeks, seed treatment and test formulation details were not described, test organisms were not described, analytical methods were not reported, statistical analysis were not described, controls were not used, environmental conditions were not adequately described, the efficacy of the endpoints and the methods for determining the endpoints were not validated, criteria for determining effects were not detailed, and tabular data was not presented to allow independent analysis. Furthermore, the study authors confirmed that concentrations of clothianidin residues measured in the guttation fluid were within the critical range corresponding to the acute LD<sub>50</sub> for up to about 40 days. Also, in the first trial, corn was treated with several clothianidin-containing test material (Mesuro, Cruiser or Gaucho) that was evidently planted at the same time and application rates or formulations were not described, confounding the results of the first trial.

The study authors presented that the “majority” of bees die in the field, not at the flight entrance; however, the percentage represented by the “majority” is not defined, nor is a reference for this detail provided. Additionally, the study authors stated that the concentrations of clothianidin residues measured in the guttation fluid were within the critical range corresponding to the acute LD<sub>50</sub> (citation provided) for up to about 40 days. Considering there were other food sources nearby (dandelion, apple trees, and clover),

combined with the majority of bees likely dying in the field (not sampled or estimated), the low bee mortality rates determined and the absence of clothianidin residues in dead bees collected at the flight entrance is not an accurate indicator of bee mortality due to exposure to clothianidin because of these confounding factors. Finally, if bees died rapidly in the fields due to clothianidin exposure, they may not have returned to the hive to contaminate the honey, and therefore clothianidin would not be found in the honey. The speed of bee death following exposure to guttation fluid within the acute LD<sub>50</sub> range was not explored or reported. Although the study author states that colony development was not affected, no specific population measurements of the test colonies were performed and therefore, colony health could not be determined.

#### **14. REFERENCES:**

Illies et al. 2002. The influence of different bee traps on undertaking behaviour of the honey bee and development of a new trap. *Apidologie* 33: 315-326.